REMARKS

Upon entry of the foregoing amendments, the specification has been amended as shown above to remove URLs from the specification. The specification also has been amended to properly identify trademarks and include the generic terminology. No new matter has been added by the amendments to the specification.

Applicants have cancelled Claims 1-3 and 9-10 without prejudice to, or disclaimer of, the subject matter contained therein. Applicants maintain that the cancellation of a claim makes no admission as to its patentability and reserve the right to pursue the subject matter of the cancelled claim in this or any other patent application.

Applicants have amended Claims 4-8 to delete reference to the Figures. Claims 4-6 are amended to delete elements (c) and (d). Claims 4 and 5 are amended to include the limitation "wherein said isolated polypeptide is more highly expressed in normal skin and esophageal tumor than in melanoma tumor and normal esophagus, respectively, or wherein said isolated polypeptide is encoded by a polynucleotide that is more highly expressed in normal skin and esophageal tumor than in melanoma tumor and normal esophagus, respectively."

Applicants have amended Claim 4 to be in independent form, and have amended Claims 5 and 12 to depend from Claim 4. Claim 13 is amended to replace the term "epitope tag" with the term "tag polypeptide." New Claims 14-17 have been added.

Applicants submit that no new matter was added by the amendments, and that support for the amendments can be found throughout the specification. Support for the amendments to Claims 4 and 5 can be found, for example, in Example 18 beginning at paragraph [0529], as well as paragraph [0336] of the specification. Support for the amendments to Claim 13 can be found, for example, at paragraph [0229]. Support for new Claims 14-17 can be found, for example, in the claims as originally filed and paragraphs [0336], [0362], [407], and Example 18 starting at paragraph [0529].

Claims 4-8, and 11-17 are presented for examination. Applicants respond below to the specific rejections raised by the PTO in the Office Action mailed January 7, 2005. For the reasons set forth below, Applicants respectfully traverse.

Correction of Inventorship under 37 CFR §1.48(b)

Applicants request that several inventors be deleted, as these inventors' inventions are no longer being claimed in the present application as a result of prosecution. The fee as set forth in § 1.17(i) is submitted herewith.

Specification

URLs:

The Examiner objected to the specification because it contains embedded hyperlinks. Applicants have amended the specification to address the Examiner's concern. In particular, Applicants have replaced the hyperlink with text that describes the location of the website. The amended text no longer constitutes browser executable code.

Trademarks:

The disclosure also was objected to by the PTO as containing trademarks which were not capitalized and did not include generic terminology. The specification has been amended to include these changes.

Rejection under 35 U.S.C. §101 – Utility

The PTO has rejected Claims 1-13 as lacking a specific, substantial, and credible utility. The PTO asserts that "[u]ses such as assaying for binding partners (p. 95), using polypeptides as molecular weight markers (p. 92), and screening for agonists and antagonists of PRO3566 (p. 95-99) are useful only in research to determine the function of the encoded protein itself." The PTO argues that there is no "specific benefit in currently available form" to be derived from such studies.

The PTO states that "[e]ven though Applicants teach that PRO3566 DNA is 'more highly expressed' in normal skin cells and esophageal tumor cells when compared to melanoma tumor cells and normal esophageal cells, respectively ... there is no guidance in the specification as to how high [the] levels are." The PTO asserts that the asserted utility in diagnosis and treatment of cancers is not substantial. The PTO argues that it is not clear whether the overexpression of PRO3566 is statistically significant and whether such overexpression is correlated to the overexpression of the claimed protein or whether it is due to aneuploidy. According to the PTO,

"the specification fails to disclose the biological significance of this overexpression." Further, according to the PTO, the specification does not teach whether the overexpression is the cause or the result of the tumors, and that the only thing Applicants teach is that the gene was more highly expressed, and this does not enable the skilled artisan to differentiate amongst expression levels in order to diagnose any diseases. Therefore, the PTO concludes that further research is required to identify or confirm a "real world" utility.

Applicants respectfully disagree.

Utility – Legal Standard

According to the Utility Examination Guidelines ("Utility Guidelines"), 66 Fed. Reg. 1092 (2001) an invention complies with the utility requirement of 35 U.S.C. § 101, if it has at least one asserted "specific, substantial, and credible utility" or a "well-established utility."

Under the Utility Guidelines, a utility is "specific" when it is particular to the subject matter claimed. For example, it is generally not enough to state that a nucleic acid is useful as a diagnostic tool without also identifying the condition that is to be diagnosed.

The requirement of "substantial utility" defines a "real world" use, and derives from the Supreme Court's holding in *Brenner v. Manson*, 383 U.S. 519, 534 (1966) stating that "The basic *quid pro quo* contemplated by the Constitution and the Congress for granting a patent monopoly is the benefit derived by the public from an invention with substantial utility." In explaining the "substantial utility" standard, M.P.E.P. § 2107.01 cautions, however, that Office personnel must be careful not to interpret the phrase "immediate benefit to the public" or similar formulations used in certain court decisions to mean that products or services based on the claimed invention must be "currently available" to the public in order to satisfy the utility requirement. "Rather, *any reasonable use that an applicant has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient,* at least with regard to defining a 'substantial' utility." (M.P.E.P. § 2107.01, emphasis added).

The mere consideration that further experimentation might be performed to more fully develop the claimed subject matter does not support a finding of lack of utility. M.P.E.P. § 2107.01 III cites *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995) in stating that "Usefulness in patent law ... necessarily includes the expectation of further research and development. The stage at which an invention in this field becomes useful is well before it is

ready to be administered to humans." Further, "[T]o violate § 101 the claimed device must be totally incapable of achieving a useful result" *Juicy Whip Inc. v. Orange Bang Inc.*, 51 USPQ2d 1700 (Fed. Cir. 1999), *citing Brooktree Corp. v. Advanced Micro Devices, Inc.*, 977 F.2d 1555, 1571 (Fed.Cir.1992).

Indeed, the Guidelines for Examination of Applications for Compliance With the Utility Requirement, set forth in M.P.E.P. § 2107 II(B)(1) gives the following instruction to patent examiners: "If the applicant has asserted that the claimed invention is useful for any particular practical purpose ... and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility."

Utility need NOT be Proved to a Statistical Certainty – a Reasonable Correlation between the Evidence and the Asserted Utility is Sufficient

An Applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement of 35 U.S.C. § 101, "unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope." *In re Langer*, 503 F.2d 1380, 1391, 183 USPQ 288, 297 (CCPA 1974). *See, also In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); *In re Irons*, 340 F.2d 974, 144 USPQ 351 (1965); *In re Sichert*, 566 F.2d 1154, 1159, 196 USPQ 209, 212-13 (CCPA 1977). Compliance with 35 U.S.C. § 101 is a question of fact. *Raytheon v. Roper*, 724 F.2d 951, 956, 220 USPQ 592, 596 (Fed. Cir. 1983) cert. denied, 469 US 835 (1984). The evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the evidence, or "more likely than not" standard. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). This is stated explicitly in the M.P.E.P.:

> [T]he applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." **Nor must the applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty**. Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. M.P.E.P. at § 2107.02, part VII (2004) (underline emphasis in original, bold emphasis added, internal citations omitted).

The PTO has the initial burden to offer evidence "that one of ordinary skill in the art would reasonably doubt the asserted utility." *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995). Only then does the burden shift to the Applicant to provide rebuttal

evidence. *Id.* As stated in the M.P.E.P., such rebuttal evidence does not need to absolutely prove that the asserted utility is real. Rather, the evidence only needs to be reasonably indicative of the asserted utility.

In *Fujikawa v. Wattanasin*, 93 F.3d 1559, 39 U.S.P.Q.2d 1895 (Fed. Cir. 1996), the Court of Appeals for the Federal Circuit upheld a PTO decision that *in vitro* testing of a novel pharmaceutical compound was sufficient to establish practical utility, stating the following rule:

> [T]esting is often required to establish practical utility. But the test results **need not absolutely prove** that the compound is pharmacologically active. All that is required is that the tests be "*reasonably* indicative of the desired [pharmacological] response." In other words, there must be **a sufficient correlation** between the tests and an asserted pharmacological activity so as to convince those skilled in the art, **to a reasonable probability**, that the novel compound will exhibit the asserted pharmacological behavior." *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1564, 39 U.S.P.Q.2d 1895 (Fed. Cir. 1996) (internal citations omitted, bold emphasis added, italics in original).

While the *Fujikawa* case was in the context of utility for pharmaceutical compounds, the principals stated by the Court are applicable in the instant case where the asserted utility is for a therapeutic and diagnostic use – utility does not have to be established to an absolute certainty, rather, the evidence must convince a person of skill in the art "to a reasonable probability." In addition, the evidence need not be direct, so long as there is a "sufficient correlation" between the tests performed and the asserted utility.

The Court in *Fujikawa* relied in part on its decision in *Cross v. Iizuka*, 753 F.2d 1040, 224 U.S.P.Q. 739 (Fed. Cir. 1985). In *Cross*, the Appellant argued that basic *in vitro* tests conducted in cellular fractions did not establish a practical utility for the claimed compounds. Appellant argued that more sophisticated *in vitro* tests using intact cells, or *in vivo* tests, were necessary to establish a practical utility. The Court in *Cross* rejected this argument, instead favoring the argument of the Appellee:

> [*I*]*n vitro* results...are generally predictive of *in vivo* test results, i.e., there is a **reasonable correlation** therebetween. Were this not so, the testing procedures of the pharmaceutical industry would not be as they are. [Appellee] has not urged, and rightly so, that there is an invariable exact correlation between *in vitro* test results and *in vivo* test results. Rather, [Appellee's] position is that successful *in vitro* testing for a particular pharmacological activity establishes a **significant probability** that *in vivo* testing for this particular pharmacological activity will be successful. *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 U.S.P.Q. 739 (Fed. Cir. 1985) (emphasis added).

The *Cross* case is very similar to the present case. Like *in vitro* testing in the pharmaceutical industry, those of skill in the field of biotechnology rely on the reasonable correlation that exists between gene expression and protein expression (see below). Were there no reasonable correlation between the two, the techniques that measure gene levels such as microarray analysis, differential display, and quantitative PCR would not be so widely used by those in the art. As in *Cross*, Applicants here do not argue that there is "an invariable exact correlation" between gene expression and protein expression. Instead, Applicants' position detailed below is that a measured change in gene expression in cancer cells establishes a "significant probability" that the expression of the encoded polypeptide in cancer will also be changed based on "a reasonable correlation therebetween."

Taken together, the legal standard for demonstrating utility is a relatively low hurdle. An Applicant need only provide evidence such that it is **more likely than not that a person of skill in the art would be convinced, to a reasonable probability, that the asserted utility is true.** The evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. The Applicant **does not need to provide evidence such that it establishes an asserted utility as a matter of statistical certainty.**

Even assuming that the PTO has met its initial burden to offer evidence that one of ordinary skill in the art would reasonably doubt the truth of the asserted utility, Applicants assert that they have met their burden of providing rebuttal evidence such that it is more likely than not those skilled in the art, to a reasonable probability, would believe that the claimed invention is useful as a diagnostic tool for cancer.

Substantial Utility

Summary of Applicants' Arguments and the PTO's Response

Applicants offer below a summary of their argument and the disputed issues involved. Applicants assert that the claimed polypeptides have utility as diagnostic tools for cancer, particularly melanoma and esophageal cancer. Applicants' asserted utility rests on the following argument:

1. Applicants have provided reliable evidence that mRNA for the PRO3566 polypeptide is more highly expressed in normal skin compared to melanoma tumor, and in esophageal tumor compared to normal esophagus;

2. Applicants assert that it is well-established in the art that a change in the level of mRNA for a particular protein, *e.g.* a decrease, generally leads to a corresponding change in the level of the encoded protein, *e.g.* a decrease;

3. Given Applicants' evidence that the level of mRNA for the PRO3566 polypeptide is decreased in melanoma tumors and in normal esophagus tissue compared to normal skin tissue and esophageal tumors, respectively, it is likely that the expression of PRO3566 polypeptide in melanoma tumors and normal esophagus tissue is also reduced, and it is therefore useful as a diagnostic tool.

Applicants understand the PTO to be making several arguments in response to Applicants' asserted utility:

1. The PTO has challenged the significance and reliability of the evidence reported in Example 18, and states that these data do not allow a skilled artisan to differentiate amongst expression levels in order to diagnose any disease;

2. The PTO asserts that it is not clear whether the overexpression of PRO3566 is statistically significant or whether such overexpression is correlated to the overexpression of the claimed protein or whether it is due to aneuploidy.

3. The PTO concludes that the data of Example 18 do not necessarily indicate anything significant regarding the claimed polypeptides. Therefore, further research needs to be done to use PRO3566 as a cancer diagnostic tool.

As detailed below, Applicants submit that the PTO has failed to meet its initial burden to offer evidence "that one of ordinary skill in the art would reasonably doubt the asserted utility." *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995). First, Applicants submit herewith a copy of a declaration of J. Christopher Grimaldi, (attached as Exhibit 1) which establishes the reliability of the data of Example 18. Knowing the biological significance of the data, or the role of PRO3566 in cancer, is not necessary to use the claimed polypeptides as cancer diagnostic tools. Second, whether or not aneuploidy is involved is irrelevant, what is important is that there are different levels of mRNA in normal cells compared to the corresponding tumor cells, which provides a utility for the differentially expressed nucleic acid and polypeptide.

Finally, even if the PTO has met its initial burden, Applicants have submitted enough rebuttal evidence such that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true. As stated above, Applicants ' evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. **The standard is not absolute or statistical certainty.**

Applicants have established that the Gene Encoding the PRO3566 Polypeptide is Differentially Expressed in Certain Cancers compared to Normal Tissue and is Useful as a Diagnostic Tool

Applicants first address the PTO's argument that the evidence of higher expression of the gene encoding the PRO3566 polypeptide in normal skin and esophageal tumor compared to melanoma tumor and normal esophagus tissue, respectively, is insufficient and unreliable. Applicants also address the PTO's statement doubting the instant utility because aneuploidy may be involved in the differential expression. Applicants submit that the gene expression data provided in Example 18 of the present application are sufficient to establish a specific and substantial utility for the claimed polypeptides.

Applicants have submitted herewith a copy of a declaration of J. Christopher Grimaldi, an expert in the field of cancer biology, originally submitted in a related co-pending and co-owned patent application Serial No. 10/063,557 (Exhibit 1). In paragraph 5 of his declaration, Mr. Grimaldi states that the gene expression studies reported in Example 18 of the instant application were made from pooled samples of normal and of tumor tissues.

In paragraphs 6 and 7, Mr. Grimaldi explains that the semi-quantitative analysis employed to generate the data of Example 18 is sufficient to determine if a gene is over- or underexpressed in tumor cells compared to corresponding normal tissue. He states that any visually detectable difference seen between two samples is indicative of at least a two-fold difference in cDNA between the tumor tissue and the counterpart normal tissue. Thus, the results of Example 18 reflect at least a two-fold difference between normal and tumor samples. He also states that the results of the gene expression studies indicate that the genes of interest "can be used to differentiate tumor from normal," thus establishing their reliability. He explains that, contrary to the PTO's assertions, "The precise levels of gene expression are irrelevant; what matters is that there is a relative difference in expression between normal tissue and tumor tissue." (Paragraph 7). Thus, since it is the relative level of expression between normal tissue

and suspected cancerous tissue that is important, the precise level of expression in normal tissue is irrelevant. Likewise, there is no need for quantitative data to compare the level of expression in normal and tumor tissue. As Mr. Grimaldi states, "If a difference is detected, this indicates that the gene and its corresponding polypeptide and antibodies against the polypeptide are useful for diagnostic purposes, to screen samples to differentiate between normal and tumor."

Applicants fail to see how whether the differential expression reported in Example 18 is due to aneuploidy or not is relevant to the utility of the disclosed nucleic acids, or their corresponding polypeptides and antibodies. Regardless of whether the differential expression of the gene encoding PRO3566 is a result of increased or decreased transcription of the gene, aneuploidy, or some other regulatory mechanism, the fact remains that it is more highly expressed in normal skin compared to melanoma tumor, and it is therefore useful as a diagnostic tool for cancer since it can be used as a molecular marker for cancer.

The fact that the PRO3566 nucleic acids and polypeptides are differentially expressed confers utility regardless of whether aneuploidy was involved. The Revised Interim Utility Guidelines promulgated by the PTO recognize that proteins which are differentially expressed in cancer have utility. (*See* the caveat in Example 12 which state that the utility requirement is satisfied where a protein is expressed in melanoma cells but not on normal skin and antibodies against the protein can be used to diagnose cancer.) In addition, while Applicants appreciate that actions taken in other applications are not binding on the PTO with respect to the present application, Applicants note that the PTO has issued several patents claiming differentially expressed polypeptides. (*See, e.g.,* U.S. Patent No. 6,414,117 and U.S. Patent No. 6,124,433, attached hereto as Exhibits 2 and 3.)

In conclusion, Applicants submit that the evidence reported in Example 18, combined with the Grimaldi Declaration submitted as Exhibit 1, establish that there is at least a two-fold difference in PRO3566 cDNA between normal skin and melanoma tumor, and esophageal tumor and normal esophagus tissue. Therefore, it follows that expression levels of the PRO3566 gene can be used to distinguish melanoma tumor tissue from normal skin, and esophageal tumor from normal esophagus tissue. The PTO has not offered any significant arguments or evidence to the contrary.

As Applicants explain below, it is more likely than not that the PRO3566 polypeptide is also differentially expressed in melanoma tumor tissue and esophageal tumor tissue, and can

therefore also be used to distinguish melanoma tumor tissue from normal skin, and esophageal tumor from normal esophagus tissue. This provides utility for the claimed polypeptides.

Applicants have established that the Accepted Understanding in the Art is that there is a Direct Correlation between mRNA Levels and the Level of Expression of the Encoded Protein

The PTO argues that it is not clear whether the overexpression of PRO3566 is correlated to the overexpression of the claimed protein or whether it is due to aneuploidy.

However, as Applicants have stated above, **whether an increase in gene copy number, for example, because of aneuploidy, leads to an increase in gene expression or protein expression is not presently an issue in this application.** The data of Example 18 reflects mRNA data as assessed by examining cDNA created from mRNA. **It is the correlation between mRNA level, as assessed by probing the cDNA library, and the level of protein expression which is at issue here, not the correlation of gene copy number and mRNA levels.** The data Applicants report in Example 18 indicate that there are more copies of the mRNA encoding PRO3566 in normal skin and esophageal tumor compared to melanoma tumor and normal esophagus, respectively. It is well-established in the art that changes in the level of mRNA are positively correlated to the changes in the level of protein.

As stated above, the standard for utility is not absolute certainty, but rather whether one of skill in the art would be more likely than not to believe the asserted utility. In fact, the working hypothesis among those skilled in the art is that there is a direct correlation between mRNA levels and protein levels.

Applicants submit herewith a copy of a second Declaration by J. Christopher Grimaldi, an expert in the field of cancer biology (attached as Exhibit 4). This declaration was submitted in connection with the related co-pending and co-owned application Serial No. 10/063,557. As stated in paragraph 5 of the declaration, "Those who work in this field are well aware that in the vast majority of cases, when a gene is over-expressed...the gene product or polypeptide will also be over-expressed.... This same principal applies to gene under-expression." Further, "the detection of increased mRNA expression is expected to result in increased polypeptide expression, and the detection of decreased mRNA expression is expected to result in decreased polypeptide expression. The detection of increased or decreased polypeptide expression can be

used for cancer diagnosis and treatment." The references cited in the declaration and submitted herewith support this statement.

Applicants also submit herewith a copy of the declaration of Paul Polakis, Ph.D. (attached as Exhibit 5), an expert in the field of cancer biology, originally submitted in a related and co-owned patent application Serial No. 10/032,996. As stated in paragraph 6 of his declaration:

> Based on my own experience accumulated in more than 20 years of research, including the data discussed in paragraphs 4 and 5 above [showing a positive correlation between mRNA levels and encoded protein levels in the vast majority of cases] and my knowledge of the relevant scientific literature, it is my considered scientific opinion that for human genes, an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell. In fact, *it remains a central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded protein.* (Emphasis added).

Dr. Polakis acknowledges that there are published cases where such a correlation does not exist, but states that it is his opinion, based on over 20 years of scientific research, that "such reports are exceptions to the commonly understood general rule that increased mRNA levels are predictive of corresponding increased levels of the encoded protein." (Polakis Declaration, paragraph 6).

The statements of Grimaldi and Polakis are supported by the teachings in Molecular Biology of the Cell, a leading textbook in the field (Bruce Alberts, *et al.*, Molecular Biology of the Cell (3rd ed. 1994) (submitted herewith as Exhibit 6) and (4th ed. 2002) (submitted herewith as Exhibit 7)). Figure 9-2 of Exhibit 6 shows the steps at which eucarotic gene expression can be controlled. The first step depicted is transcriptional control. Exhibit 6 provides that "[f]or <u>most genes</u> transcriptional controls are paramount. This makes sense because, of all the possible control points illustrated in Figure 9-2, only transcriptional control ensures that no superfluous intermediates are synthesized." Exhibit 6 at 403 (emphasis added). In addition, the text states that "Although <u>controls on the initiation of gene transcription are the predominant form of regulation for most genes</u>, other controls can act later in the pathway from RNA to protein to modulate the amount of gene product that is made." Exhibit 6 at 453 (emphasis added). Thus, as established in Exhibit 6, the predominant mechanism for regulating the amount of protein produced is by regulating transcription initiation.

In Exhibit 7, Figure 6-3 on page 302 illustrates the basic principle that there is a correlation between increased gene expression and increased protein expression. The accompanying text states that "a cell can change (or regulate) the expression of each of its genes according to the needs of the moment – *most obviously by controlling the production of its mRNA*." Exhibit 7 at 302 (emphasis added). Similarly, Figure 6-90 on page 364 of Exhibit 7 illustrates the path from gene to protein. The accompanying text states that while potentially each step can be regulated by the cell, "the initiation of transcription is the most common point for a cell to regulate the expression of each of its genes." Exhibit 7 at 364 (emphasis added). This point is repeated on page 379, where the authors state that of all the possible points for regulating protein expression, "[f]or most genes transcriptional controls are paramount." Exhibit 7 at 379 (emphasis added).

Further support for Applicants' position can be found in the textbook, Genes VI, (Benjamin Lewin, Genes VI (1997)) (submitted herewith as Exhibit 8) which states "having acknowledged that control of gene expression can occur at multiple stages, and that production of RNA cannot inevitably be equated with production of protein, it is clear that the overwhelming majority of regulatory events occur at the initiation of transcription." *Genes VI* at 847-848 (emphasis added).

Additional support is also found in Zhigang *et al.*, World Journal of Surgical Oncology 2:13, 2004, submitted herewith as Exhibit 9. Zhigang studied the expression of prostate stem cell antigen (PSCA) protein and mRNA to validate it as a potential molecular target for diagnosis and treatment of human prostate cancer. The data showed "a high degree of correlation between PSCA protein and mRNA expression" Exhibit 9 at 4. Of the samples tested, 81 out of 87 showed a high degree of correlation between mRNA expression and protein expression. The authors conclude that "it is demonstrated that PSCA protein and mRNA overexpressed in human prostate cancer, and that the increased protein level of PSCA was resulted from the upregulated transcription of its mRNA." Exhibit 9 at 6. Even though the correlation between mRNA expression and protein expression occurred in 93% of the samples tested, not 100%, the authors state that "PSCA may be a promising molecular marker for the clinical prognosis of human Pca and a valuable target for diagnosis and therapy of this tumor." Exhibit 9 at 7.

Further, Meric *et al.*, Molecular Cancer Therapeutics, vol. 1, 971-979 (2002), submitted herewith as Exhibit 10, states the following:

> The **fundamental principle** of molecular therapeutics in cancer is to exploit the differences in gene expression between cancer cells and normal cells...[M]ost efforts have concentrated on identifying differences in gene expression at the level of mRNA, which can be attributable to either DNA amplification or to differences in transcription. Meric *et al.* at 971 (emphasis added).

Those of skill in the art would not be focusing on differences in gene expression between cancer cells and normal cells if there were no correlation between gene expression and protein expression.

Together, the declarations of Grimaldi and Polakis, the accompanying references, and the excerpts and references provided above all establish that the accepted understanding in the art is that there is a reasonable correlation between changes in gene expression and the level of the encoded protein. In light of the lack of support for any argument by the PTO to the contrary, Applicants submit that they have established that it is more likely than not that one of skill in the art would believe that because the PRO3566 mRNA is expressed at a higher level in normal skin and esophageal tumor compared to melanoma tumor and normal esophagus, respectively, the PRO3566 polypeptide will also be expressed at a higher level in normal skin and esophageal tumor compared to melanoma tumor and normal esophagus, respectively. One of skill in the art would recognize that a protein which is differentially expressed in certain cancer cells compared to the corresponding normal tissue would have utility as a diagnostic tool. Thus, Applicants submit that they have established that it is more likely than not that one of skill in the art would recognize the asserted utility of the claimed polypeptides as a cancer diagnostic tool.

The Claimed Polypeptide would have Diagnostic Utility even if there is no Positive Correlation between Gene Expression and Expression of the Encoded Polypeptide

Even assuming *arguendo* that, there is no direct correlation between changes in gene expression and changes in protein expression for PRO3566, which Applicants submit is not true, a polypeptide encoded by a gene that is differentially expressed in cancer would **still** have a credible, specific and substantial utility.

In paragraph 6 of the second Grimaldi Declaration, Exhibit 4, Mr. Grimaldi explains that:

> However, even in the rare case where the protein expression does not correlate with the mRNA expression, this still provides significant information useful for cancer diagnosis and treatment. For example, if over- or under-expression of a

> gene product does not correlate with over- or under-expression of mRNA in certain tumor types but does so in others, then identification of both gene expression and protein expression enables more accurate tumor classification and hence better determination of suitable therapy.

This conclusion is echoed in the Declaration of Avi Ashkenazi, Ph.D. (attached as Exhibit 11), an expert in the field of cancer biology. This declaration was previously submitted in connection with co-pending application Serial No. 09/903,925. Applicants submit that simultaneous testing of gene expression and gene product expression enables more accurate tumor classification, even if there is no positive correlation between the two. This leads to better determination of a suitable therapy.

This is further supported by the teachings in the article by Hanna and Mornin, submitted herewith (attached as Exhibit 12). The article teaches that the HER-2/neu gene has been shown to be amplified and/or over-expressed in 10%-30% of invasive breast cancers and in 40-60% of intraductal breast carcinoma. Further, the article teaches that diagnosis of breast cancer includes testing both the amplification of the HER-2/neu gene (by FISH) as well as the overexpression of the HER-2/neu gene product (by IHC). Even when the protein is not overexpressed, the assay relying on both tests leads to a more accurate classification of the cancer and a more effective treatment of it.

The Applicants have established that it is the general, accepted understanding in the art that there is a positive correlation between gene expression and protein expression. However, even when this is not the case, a polypeptide encoded by a gene that is differentially expressed in cancer would still have utility. Thus, Applicants have demonstrated another basis for supporting the asserted utility for the claimed polypeptides.

The Arguments made by the PTO are Not Sufficient to satisfy the PTO's Initial Burden of Offering Evidence "that one of ordinary skill in the art would reasonably doubt the asserted utility"

As stated above, an Applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement of 35 U.S.C. § 101, "unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope." *In re Langer*, 503 F.2d 1380, 1391, 183 USPQ 288, 297 (CCPA 1974). The evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the

evidence, or "more likely than not" standard. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). This is stated explicitly in the M.P.E.P.:

> [T]he applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." **Nor must the applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty**. Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not. M.P.E.P. at § 2107.02, part VII (2004) (underline emphasis in original, bold emphasis added, internal citations omitted).

The PTO has the initial burden to offer evidence "that one of ordinary skill in the art would reasonably doubt the asserted utility." *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995). Only then does the burden shift to the Applicant to provide rebuttal evidence. *Id.* As stated in the M.P.E.P., such rebuttal evidence does not need to absolutely prove that the asserted utility is real. Rather, the evidence only needs to be reasonably indicative of the asserted utility.

The PTO has not offered any arguments or cited any references to establish "that one of ordinary skill in the art would reasonably doubt" that the disclosed polypeptide is differentially expressed in certain tumors and that the claimed polypeptides can be used as diagnostic tools. Given the lack of support for the PTO's position, Applicants submit that the PTO has not met its initial burden of overcoming the presumption that the asserted utility is sufficient to satisfy the utility requirement. And even if the PTO has met that burden, the Applicants' supporting rebuttal evidence is sufficient to establish that one of skill in the art would be more likely than not to believe that the claimed polypeptides can be used as diagnostic tools for cancer, particularly skin cancer.

Specific Utility

The Asserted Substantial Utilities are Specific to the Claimed Polypeptides

Specific Utility is defined as utility which is "specific to the subject matter claimed," in contrast to "a general utility that would be applicable to the broad class of the invention." M.P.E.P. § 2107.01 I. Applicants submit that the evidence of differential expression of the PRO3566 gene in certain types of cancer cells, along with the declarations and references discussed above, provide a specific utility for the claimed polypeptides.

As discussed above, there are significant data which show that the gene encoding the PRO3566 polypeptide is more highly expressed in normal skin and in esophageal tumor compared to melanoma tumor and normal esophagus, respectively. These data are strong evidence that the PRO3566 polypeptide is associated with melanoma and esophageal tumors. Thus, contrary to the assertions of the PTO, Applicants submit that they have provided evidence associating the PRO3566 polypeptide with a specific disease. Use of the claimed polypeptides as a diagnostic tool for cancer, particularly melanoma and esophageal tumors, is a specific utility – it is not a general utility that would apply to the broad class of polypeptides.

Conclusion

The PTO has asserted two arguments for why there is a lack of a substantial utility: (1) that the data reporting differential expression of the PRO3566 gene in certain cancers is not significant and reliable; and, (2) that because there is no necessary correlation between gene amplification and protein expression, the claimed polypeptides cannot be used as cancer diagnostic or therapeutic tools. Applicants have addressed each of these arguments in turn.

First, the Applicants provided a first Declaration of Chris Grimaldi stating that the data in Example 18 are real and significant. This declaration also indicates that given the at least two-fold difference in expression levels, the disclosed nucleic acids and corresponding polypeptides have utility as cancer diagnostic tools. Applicants have demonstrated that it is not necessary to know the cause or consequence of the differential expression of PRO3566 nucleic acids and polypeptides in melanoma and esophageal tumors in order to use them as diagnostic tools for cancer.

Next, Applicants submit that the second Grimaldi Declaration and Polakis Declaration, the accompanying references, as well as the excerpts and references cited above, demonstrate that it is well-established in the art that a change in mRNA levels generally correlates to a corresponding change in the encoded protein levels. The PTO has not offered any substantial reasoning or evidence to the contrary. One of skill in the art will recognize that polypeptides differentially expressed in certain cancers have utility as diagnostic tools for cancer.

Finally, the PTO asserts that there is no asserted specific utility. Applicants have pointed out that the substantial utilities described above are specific to the claimed polypeptides because the PRO3566 gene and polypeptide are differentially expressed in melanoma and esophageal

tumors compared to normal skin and esophagus tissue. This is not a general utility that would apply to the broad class of polypeptides.

Given the totality of the evidence provided, Applicants submit that they have established a substantial, specific, and credible utility for the claimed polypeptides as diagnostic tools. According to the PTO Utility Examination Guidelines (2001), irrefutable proof of a claimed utility is not required. Rather, a specific, substantial, and credible utility requires only a "reasonable" confirmation of a real world context of use. Applicants remind the PTO that:

> A small degree of utility is sufficient . . . The claimed invention must only be capable of performing some beneficial function . . . An invention does not lack utility merely because the particular embodiment disclosed in the patent lacks perfection or performs crudely . . . A commercially successful product is not required . . . Nor is it essential that the invention accomplish all its intended functions . . . or operate under all conditions . . . partial success being sufficient to demonstrate patentable utility . . . In short, **the defense of non-utility cannot be sustained without proof of total incapacity**. If an invention is only partially successful in achieving a useful result, a rejection of the claimed invention as a whole based on a lack of utility is not appropriate. M.P.E.P. at 2107.01 (underline emphasis in original, bold emphasis added, citations omitted).

Applicants submit that they have established that it is more likely than not that one of skill in the art would reasonably accept the utility for the claimed polypeptides relating to PRO3566 set forth in the specification. In view of the above, Applicants respectfully request that the PTO reconsider and withdraw the utility rejection under 35 U.S.C. §101.

Rejections under 35 U.S.C. § 112, second paragraph – Indefiniteness

The PTO has rejected Claims 1-6, 10 and 12-13 under 35 U.S.C. § 112, second paragraph, as being indefinite. The PTO objects to the phrase "the extracellular domain ... lacking its associated signal sequence" as a signal sequence is not generally considered to be part of the extracellular domain, as signal sequences are cleaved from said domains in the process of maturation.

Applicants have canceled Claims 1-3, and 9-10, and amended Claims 4-6 to delete reference to an extracellular domain, rendering this rejection moot. Thus, Applicants request that the PTO reconsider and withdraw the indefiniteness rejection under 35 U.S.C. §112, second paragraph.

Rejection under 35 U.S.C. §112, first paragraph – Enablement

The PTO rejected Claims 1-13 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to use the invention. The PTO argues that because the claimed invention is not supported by a substantial, specific and credible utility, the claims are not enabled.

Applicants submit that in the discussion of the 35 U.S.C. § 101 rejection above, Applicants have established a substantial, specific, and credible utility for the claimed polypeptides. Applicants therefore request that the PTO reconsider and withdraw the enablement rejection under 35 U.S.C. § 112, first paragraph, based on a lack of utility.

The PTO also has stated that even if the specification taught how to use the PRO3566 polypeptide, enablement would not be commensurate in scope with Claims 1-5 and 12-13, which encompass percentage variants of SEQ ID NO: 64. The PTO argues that there are no functional limitations in the claims, and that there is no function known in the art to be associated with PRO3566.

As amended, the pending claims are related to isolated polypeptides having at least 95% or 99% amino acid sequence identity to several polypeptides related to SEQ ID NO: 64, and which satisfy the limitation "wherein said isolated polypeptide is more highly expressed in normal skin and esophageal tumor than in melanoma tumor and normal esophagus, respectively, or wherein said isolated polypeptide is encoded by a polynucleotide that is more highly expressed in normal skin and esophageal tumor than in melanoma tumor and normal esophagus, respectively" or "wherein said isolated polypeptide or a fragment thereof can be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO: 64 in skin tissue or esophagus tissue samples."

Applicants submit that the claimed polypeptides are enabled, as one of skill in the art would know how to make and use them. Applicants submit that it is well-established in the art how to make polypeptides which have at least 95% or 99% amino acid sequence identity to the disclosed sequences related to SEQ ID NO: 64. Applicants have disclosed how to determine if the claimed polypeptides or encoding nucleic acids are differentially expressed in melanoma tumors or esophageal tumors compared to normal skin or normal esophagus, respectively. Applicants have also disclosed how to make antibodies to the polypeptide of SEQ ID NO: 64, and given the high amino acid sequence homology of the claimed polypeptides, one of skill in

the art would know how to make antibodies to SEQ ID NO: 64 from the claimed polypeptides. Thus, one of skill in the art would know how to make the claimed polypeptides.

As discussed above, Applicants submit that they have established that one of skill in the art would believe that it is more likely than not that the PRO3566 gene and polypeptide are differentially expressed in melanoma and esophageal tumors such that they can be used as cancer diagnostic tools. Given the disclosure in the specification and the level of skill in the art, a skilled artisan would know how to use the claimed polypeptides as diagnostic tools. For example, polypeptides which have at least 95% or 99% amino acid sequence identity to the disclosed sequences and are "more highly expressed in normal skin and esophageal tumor than in melanoma tumor or normal esophagus..." can be used as diagnostic tools since the claimed polypeptides or their encoding nucleic acids are differentially expressed in melanoma and esophageal tumors. Other claimed polypeptides which have at least 95% or 99% amino acid sequence identity to the disclosed sequences and "said isolated polypeptide or a fragment thereof can be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO: 64 in skin tissue or esophagus tissue samples," are also useful diagnostic tools. Because the polypeptide of SEQ ID NO: 64 is most likely differentially expressed in melanoma tumors and in esophageal tumors, antibodies for specific detection of this polypeptide in skin tissue samples and esophagus tissue samples are useful diagnostic tools.

Given the skill in the art and the disclosure of how to make and use the claimed polypeptides, Applicants request that the PTO reconsider and withdraw its rejection under 35 U.S.C. § 112, first paragraph.

Rejection under 35 U.S.C. §112, first paragraph – Written Description

The PTO has rejected Claims 1-5 and 12-13 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the invention. According to the PTO, because the claims do not require that the claimed polypeptides possess any particular biological activity, particular conserved structure, or other disclosed distinguishing feature, the claims fail the written description requirement. The PTO states that the only factor present in the claim is a partial structure in the form of a recitation of percent identity. The PTO concludes that in the absence of sufficient

recitation of distinguishing identifying characteristics, the specification does not provide adequate written description of the claimed genus.

The Legal Standard for Written Description

The well-established test for sufficiency of support under the written description requirement of 35 U.S.C. §112 , first paragraph is whether the disclosure "reasonably conveys to artisan that the inventor had possession at that time of the later claimed subject matter." *In re Kaslow*, 707 F.2d 1366, 1375, 2121 USPQ 1089, 1096 (Fed. Cir. 1983); *see also Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at1563, 19 USPQ2d at 1116 (Fed. Cir. 1991). The adequacy of written description support is a factual issue and is to be determined on a case-by-case basis. *See e.g., Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116 (Fed. Cir. 1991). The factual determination in a written description analysis depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure. *Union Oil v. Atlantic Richfield Co.*, 208 F.3d 989, 996 (Fed. Cir. 2000).

The Current Invention is Adequately Described

As noted above, whether the Applicants were in possession of the invention as of the effective filing date of an application is a factual determination, reached by the consideration of a number of factors, including the level of knowledge and skill in the art, and the teaching provided by the specification. The inventor is not required to describe every single detail of his/her invention. An applicant's disclosure obligation varies according to the art to which the invention pertains. The present invention pertains to the field of recombinant DNA/protein technology. It is well-established that the level of skill in this field is very high since a representative person of skill is generally a Ph.D. scientist with several years of experience. Accordingly, the teaching imparted in the specification must be evaluated through the eyes of a highly skilled artisan as of the date the invention was made.

As amended, the pending claims are related to isolated polypeptides having at least 95% or 99% amino acid sequence identity to several polypeptides related to SEQ ID NO: 64, and satisfy the limitation "wherein said isolated polypeptide is more highly expressed in normal skin and esophageal tumor than in melanoma tumor and normal esophagus, respectively, or wherein said isolated polypeptide is encoded by a polynucleotide that is more highly expressed in normal

skin and esophageal tumor than in melanoma tumor and normal esophagus, respectively" or "wherein said isolated polypeptide or a fragment thereof can be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO: 64 in skin tissue or esophagus tissue samples."

Applicants maintain that there is no substantial variation within the species which fall within the scope of the amended claims, which require at least 95% or 99% amino acid sequence identity to the disclosed sequences related to SEQ ID NO: 64. Applicants note that the pending Claims are analogous to the claims discussed in Example 14 of the written description training materials. In Example 14, the written description requirement was found to be satisfied for claims relating to polypeptides having 95% homology to a particular sequence and possessing a particular catalytic activity, even though the applicant had not made any variants. Similarly, the pending claims also have very high sequence homology to the disclosed sequences and must share the same expression pattern in certain tumors, or share an epitope sufficient to generate antibodies which specifically detect the polypeptide of SEQ ID NO: 64 in skin tissue or esophagus tissue samples.

In Example 14, the procedures for making variants were known in the art and the disclosure taught how to test for the claimed catalytic activity. Similarly, in the instant application, it is well known in the art how to make polypeptides with at least 95% amino acid sequence identity to the disclosed sequences. In addition, the specification discloses how to test to determine if the polypeptide or encoding nucleic acid is differentially expressed in skin tumors or esophageal tumors, and how to make antibodies which specifically detect the polypeptide of SEQ ID NO: 64 in skin tissue and esophagus tissue samples. Like Example 14, the genus of polypeptides that have at least 95% or 99% amino acid sequence identity to the disclosed sequences will not have substantial variation.

Furthermore, while Applicants appreciate that actions taken by the PTO in other applications are not binding with respect to the examination of the present application, Applicants note that the PTO has issued many patents containing claims to variant nucleic acids or variant proteins where the applicants did not actually make such nucleic acids or proteins. Representative patents include U.S. Patent No: 6,737,522, U.S. Patent No. 6,395,306, U.S. Patent No. 6,025,156, U.S. Patent No. 6,645,499, U.S. Patent No. 6,498,235, and U.S. Patent No. 6,730,502, which are attached hereto as Exhibits 13-18.

In conclusion, Applicants submit that they have satisfied the written description requirement for the pending claims based on the actual reduction to practice of SEQ ID NO: 64, by specifying a high level of amino acid sequence identity, by describing how to test for differential expression of the polypeptide and encoding nucleic acid, and by describing how to make antibodies to the disclosed sequence, all of which result in a lack of substantial variability in the species falling within the scope of the instant claims. Applicants submit that this disclosure would allow one of skill in the art to "recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus." Hence, Applicants respectfully request that the PTO reconsider and withdraw the written description rejection under 35 U.S.C. §112.

Rejection under 35 U.S.C. §102 – Anticipation

The PTO rejects Claims 1-5 as anticipated under 35 U.S.C. § 102(a) by Oka *et al.* (NCBI Accession No. BAA88132) (hereinafter Oka), which was published December 8, 1999. The PTO states that Oka discloses an amino acid sequence that has 99% identity to SEQ ID NO: 64 of the instant invention, and therefore anticipates Claims 1-5. The PTO also rejects Claims 1-4 as anticipated under 35 U.S.C. § 102(b) by Janer *et al.* (NCBI Accession No. AC006163) (hereinafter Janer), which was submitted on December 8, 1998. The PTO states that Janer discloses a nucleic acid sequence which encodes a polypeptide that is 98% identical to SEQ ID NO: 64 of the instant invention, and therefore anticipates Claims 1-4.

Applicants respectfully traverse.

Janer et al.:

Janer discloses the results of a large scale sequence analysis of the human MHC class I region, in which a nucleotide sequence of 44,379 bases is disclosed. Janer does not provide, for example, any information regarding coding regions or regarding introns. Janer does not provide the amino acid sequence of SEQ ID NO:64 or any other amino acid sequence. The PTO asserts that the 44 kilobase sequence of Janer includes a nucleotide sequence that encodes a polypeptide that is 98% identical to SEQ ID NO: 64. Applicants respectfully disagree.

As mentioned above, Janer does not provide the amino acid sequence of SEQ ID NO: 64. Janer does not describe the coding region of the sequence that encodes SEQ ID NO: 64. The

sequence disclosed by Janer includes a nucleotide sequence (presumably an intron) that interrupts the coding region of the sequence that is asserted to encode SEQ ID NO: 64. However, Janer does not recognize the intron or disclose its location. Without more information, particularly the information regarding the necessary removal of the interrupting sequence, Janer does not disclose a nucleic acid sequence that encodes SEQ ID NO: 64. Therefore, Janer does not disclose a sequence that encodes a polypeptide that is 99% identical, for example, to the amino acid sequence of the polypeptide of SEQ ID NO: 64; or to the amino acid sequence of the polypeptide of SEQ ID NO: 64 lacking its associated signal peptide. For these reasons, Janer does not teach a 95% variant according to the claims.

Oka et al.:

The PTO has rejected Claims 1-5 under 35 U.S.C. § 102(a) as being anticipated by Oka. Attached herewith is the Declaration of Audrey Goddard, Paul J. Godowski, J. Christopher Grimaldi, Austin L. Gurney and William I. Wood under 37 C.F.R. §1.131 (referred to hereafter as "the Declaration of Goddard et al."), which establishes that the presently claimed invention antedates the publication date of Oka. The Declaration of Goddard et al. establishes that the presently claimed subject matter was conceived prior to the publication date of Oka, December 8, 1999, and diligently reduced to practice on a date after the submission date of Oka. Thus, Applicants respectfully submit that the cited reference is not available as prior art, and request that the rejections under 35 USC §102(a) be withdrawn.

As set forth in 37 C.F.R. § 1.131, a patent applicant "may submit an appropriate oath or declaration to establish invention of the subject matter of the rejected claim prior to the effective date of the reference or activity on which the rejection is based." *See also*, M.P.E.P. § 715. "The affidavit or declaration must state FACTS and produce such documentary evidence and exhibits in support thereof as are available to show conception and completion of the invention in this country ... at least conception being at a date prior to the effective date of the reference." *See* M.P.E.P. § 715.07 (emphasis in original). The showing of facts must be sufficient to show "conception of the invention prior to the effective date of the reference coupled with due diligence from prior to the reference date to a subsequent (actual) reduction to practice." *See id.*

Oka was published on December 8, 1999. Oka is cited as a 102(a) reference because it allegedly discloses an amino acid sequence that is 99% identical to the sequence of SEQ ID

NO: 64. However, as set forth below, Applicants were in possession of SEQ ID NO: 64 prior to the submission date of Oka.

The Declaration and attached Exhibit A demonstrate that the claimed subject matter, particularly a polypeptide having the sequence of SEQ ID NO: 64, was conceived by Applicants prior to December 8, 1999. Furthermore, as evidenced by the Declaration and Exhibit B, Applicants exhibited diligence in reducing the subject matter of the claims to practice from at least just prior to the submission date of Oka, by performing various assays to confirm the function of the polypeptide.

In view of the above, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. §102.

CONCLUSION

In view of the above, Applicants respectfully maintain that claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: April 7, 2005

By: 

Marc T. Morley
Registration No. 52,051
Attorney of Record
Customer No. 30,313
(619) 235-8550

1434216_1
032205

>Monday, March 21, 2005
>DNA59844 [Full]
>685 Sites [All Sites]

[DNA59844], avagts (oligo)
[DNA59844], sheldens

> Lib309

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                                                 taiI
                                                snaBI
                                          nlaIII thaI maeII/hpyCH4IV
                                         sphI    fnuDII/mvnI              bcgI              thaI
                                         nspHI   bstUI                    taqI              fnuDII/mvnI
                                      hpy99I hpyCH4V rsaI                 xhoI              bstUI
                                      taiI nspI    bsh1236I               tliI       hpyCH4V bsh1236I         hinPI
                                     hinlI/acyI cac8I  csp6I         tsp509I[M.ecoRI-] sfaNI     aciI           tseI     mnlI
                 sapI aluI  ahaII/bsaHI   mluI bsaAI       ecoRI    smlI     fokI      fnu4HI/bsoFI    fnu4HI/bsoFI
                 mboII      aatII  cac8I  aflIII     aluI  apoI     paeR7I   bstF5I   haeIII/palI     aluI hhaI/cfoI
          sfcI earI/ksp632I maeII/hpyCH4IV bsiWI/splI  hpy188I     avaI[M.taqI-]    sau96I[M.haeIII-] bbvI   bseRI
1 GACACTATAG AAGAGCTATG ACGTCGCATG CACGCGTACG TAAGCTCGGA ATTCGGCTCG AGCAGGATGC AGGGCCGCGT GGCAGGGAGC TGCGCTCCTC
  CTGTGATATC TTCTCGATAC TGCAGCGTAC GTGCGCATGC ATTCGAGCCT TAAGCCGAGC TCGTCCTACG TCCCGGCGCA CCGTCCCTCG ACGCGAGGAG
1                                                                                  M  Q  G  R  V  A  G  S  C  A  P  L
                                                                                 ^insert starts here
                                                                                   ^MET
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59844.AV393.f, 5tag=TTTTTTGAATTCTAAACCAAC^

EXHIBIT A

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                ecoRII[dcm-]         pspGI
                dsaV[dcm-]           mvaI              bsaXI
                bstNI                ecoRII[dcm-]      mspI
                bssKI[dcm-]          dsaV[dcm-]        hpaII                                    sau96I
          bslI[dcm-]      mboII      bstNI             scrFI[M.hpaII-]                          avaII        dd
        cac8I   apyI[dcm+]           bssKI[dcm-]       nciI                                     nlaIV        bs
     haeIII/palI      bpuAI         apyI[dcm+]        dsaV                mnlI                 bsmFI        mnl
    sau96I[M.haeIII-] bbsI          bsaJI  mnlI       bssKI sfaNI         bseRI                hpy188III    bsl
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    ACCCGGACGA GGACCAGACA GAAGTAGAGG GTCCGGAGAA ACGGGCCTCG TAGCCACAAC ACCTCCTCTT TCAAAGGGTT TTGAAGCCCT GGTTGAACGG
 13   G   L   L   L   V   C   L   H   L   P   G   L   F   A   R   S   I   G   V   V   E   E   K   V   S   Q   N   F   G   T   N   L   P
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                        nlaIV                    fnu4HI/bsoFI sau96I                           bl
                        haeIII/palI          fokI bbvI  bsrBI nlaIV                         eco57I
      hpy188I          bsrI                  bstF5I     aciI  avaII                       mnlI  alu
    aluI            mnlI tspRI  mnlI     hpy188I  aciI  cac8I hpy188III               nlaIV  hpy188I
201 TCAGCTCGGA CAACCTTCCT CCACTGGCCC CTCTAACTCT GAACATCCGC AGCCCGCTCT GGACCCTAGG TCTAATGACT TGGCAAGGGT TCCTCTGAAG
    AGTCGAGCCT GTTGGAAGGA GGTGACCGGG GAGATTGAGA CTTGTAGGCG TCGGGCGAGA CCTGGGATCC AGATTACTGA ACCGTTCCCA AGGAGACTTC
46  Q  L  G   Q  P  S  S   T  G  P   S  N  S   E  H  P  Q   P  A  L   D  P  R   S  N  D  L   A  R  V   P  L  K

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                                                                                             pspGI
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                          sfcI                                                        nlaIII ecoRII[dcm
                         sse8387I                                                    styI    dsaV[dcm-]
                         sbfI             tspRI                                      ncoI    bstNI   bs
               pflMI     ecoNI           btsI  mwoI                             cac8I dsaI tfiI bssKI[dcm-
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 ddeI[M.aluI-]          bspMI          sfcI  hpyCH4V      mnlI            fnu4HI/bsoFI hinfI   haeIII/
 bspCNI   mnlI   hpy188I     bslI pstI  mnlI   pstI bsgI  mnlI haeIII/palI  bbvI     bsaJI     apyI[dcm+]
301 CTCAGCGTGC CTCCATCAGA TGGCTTCCCA CCTGCAGGAG GTTCTGCAGT GCAGAGGTGG CCTCCATCGT GGGGGCTGCC TGCCATGGAT TCCTGGCCCC
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79  L  S  V  P   P  S  D   G  F  P   P  A  G  G   S  A  V   Q  R  W   P  P  S  W   G  L  P   A  M  D   S  W  P  P
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      dpnI[dam+]                              ecoRII[dcm-]
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     bstYI/xhoII          fnu4HI/bsoFI       bstNI        fnu4HI/bsoFI                          fnu4HI/bsoFI
     bamHI                bbvI       avaII   bssKI[dcm-]  bbvI                                tseI  sau96I[M.h
     alwI[dam-]         tseI       ddeI      bsaJI      hinPI                                 fnu4HI/bsoFI
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  bspCNI  bsaJI         bbvI  aciI bspCNI  aciI         afeI/eco47III             mnlI  bsrI bbvI  haeIII/pal
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113   E  D  P  W  Q  M   M  A  A  A   A  E  D   R  L  G   E  A  L  P   E  E  L   S  Y  L   S  S  A  A   A  L  A
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     mspI           bsaJI  pleI                                 bspCNI       mnlI    bstNI   hpy188I
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     bslI tspRI   bslI[dcm-]   hinfI    hpy188III    stuI    hphI  mnlI      bseRI    apyI[dcm+] hinfI
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146  P  G  S   G  P  L  P   G  E  S   S  P  D   A  T  G  L   S  P  E   A  S  L   L  H  Q  D   S  E  S   R  R  L
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                                                                                                                        mvaI   rsaI
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                           hpaII                                       dsaV[dcm-]                                   sau3AI[M.hphI-]
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                  bsrI     bssKI                                       bssKI[dcm-]                                  dpnII[dam-]  banI
                 tspRI     bsaJI                                      bsaJI        fokI                             dpnI[dam+]   asp718
             tsp509I   nlaIV                                      mnlI apyI[dcm+] bstF5I                           bclI[dam-]   acc65I
601 CCCCGTTCTA ATTCACTGGG AGCCGGGGGA AAAATCCTTT CCCAACGCCC TCCCTGGTCT CTCATCCACA GGGTTCTGCC TGATCACCCC TGGGGTACCC
    GGGGCAAGAT TAAGTGACCC TCGGCCCCCT TTTTAGGAAA GGGTTGCGGG AGGGACCAGA GAGTAGGTGT CCCAAGACGG ACTAGTGGGG ACCCCATGGG
179 P  R  S  N   S  L  G   A  G  G   K  I  L  S   Q  R  P   P  W  S   L  I  H  R   V  L  P   D  H  P   W  G  T  L
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                                          bsmFI
                                        scrFI[dcm-]
                                        pspGI
                                        mvaI
                                        ecoRII[dcm-]
                                        dsaV[dcm-]
                        scrFI[dcm-]     bstNI
                        pspGI           bssKI[dcm-]
                        mvaI            bslI[dcm-]
                        ecoRII[dcm-]    bsaJI
                        dsaV[dcm-]      apyI[dcm+]                          ddeI
                        bstNI         sau96I[dcm-][M.haeIII-]    sau96I[M.haeIII-]  mnlI
                        bssKI[dcm-]   haeIII/palI                haeIII/palI     bspCNI tfiI
    tfiI bsrI           bsaJI  mnlI   eco0109I/draII[dcm-]      mnlI            eco81I  hinfI
    hinfI tspRI         apyI[dcm+]   mnlI bsaJI  bsrI          bcgI   nlaIII     bsu36I/mstII/sauI
701 TGAATCCCAG TGTGTCCTGG GGAGGTGGAG GCCCTGGGAC TGGTTGGGGA ACGAGGCCCA TGCCACACCC TGAGGGAATC TGGGGTATCA ATAATCAACC
    ACTTAGGGTC ACACAGGACC CCTCCACCTC CGGGACCCTG ACCAACCCCT TGCTCCGGGT ACGGTGTGGG ACTCCCTTAG ACCCCATAGT TATTAGTTGG
213   N  P  S   V  S  W   G  G  G  G   P  G  T   G  W  G   T  R  P  M   P  H  P   E  G  I   W  G  I  N   N  Q  P
```

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        rsaI
        csp6I
       nlaIV
       kpnI
       banI
       asp718[dcm-]
       acc65I[dcm-]                    xcmI      aluI                    xcmI     pvuII[M.HI-]
    xcmI                               scrFI[dcm-]                       scrFI[dcm-]
    scrFI[dcm-]                        pspGI    pvuII[M.H1-]             pspGI    mspA1I/nspBII
    pspGI                              mvaI    tseI                      mvaI    tseI
    mvaI      aluI                     ecoRII[dcm-]                      ecoRII[dcm-]
    ecoRII[dcm-]                       dsaV[dcm-]                        dsaV[dcm-]
    dsaV[dcm-]                         bstNI   fnu4HI/bsoFI              bstNI   fnu4HI/bsoFI
    bstNI   pvuII                      bssKI[dcm-]             tru9I     bssKI[dcm-]             tru9I
    bssKI[dcm-]             tru9I      apyI[dcm+]              mseI      apyI[dcm+]              mseI
    apyI[dcm+]         sspI mseI    bciVI       bbvI        sspI        bciVI       bbvI           aseI/asnI/vspI
   bsaJI    mspA1I/nspBII  aseI/asnI/vspI   mnlI mspA1I/nspBII aseI/asnI/vspI    mnlI aluI      sspI        bciVI
801 CCCAGGTACC AGCTGGGGAA ATATTAATCG GTATCCAGGA GGCAGCTGGG GAAATATTAA TCGGTATCCA GGAGGCAGCT GGGGGAATAT TAATCGGTAT
    GGGTCCATGG TCGACCCCTT TATAATTAGC CATAGGTCCT CCGTCGACCC CTTTATAATT AGCCATAGGT CCTCCGTCGA CCCCCTTATA ATTAGCCATA
246  P  G  T   S  W  G  N   I  N  R   Y  P  G   G  S  W  G   N  I  N   R  Y  P   G  G  S  W   G  N  I   N  R  Y
```

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          pvuII[M.H1-]
       mnlI aluI                    scrFI[dcm-]              bpmI/gsuI[dcm-]
    xcmI    mspA1I/nspBII           pspGI                    scrFI[dcm-]        scrFI[dcm-]
    scrFI[dcm-]                     mvaI                     pspGI              pspGI
    pspGI  tseI                     ecoRII[dcm-]             mvaI               mvaI   mboII
    mvaI   fnu4HI/bsoFI             dsaV[dcm-]               ecoRII[dcm-]       ecoRII[dcm-]
    ecoRII[dcm-]                    bstNI                    dsaV[dcm-]         dsaV[dcm-]
    dsaV[dcm-]                      bssKI[dcm-]              bstNI          mwoI bstNI earI/ksp632I      alu
    bstNI  bbvI                     apyI[dcm+]               bssKI[dcm-]    bglI bssKI[dcm-]            pvuI
    bssKI[dcm-]                     bsaJI                    apyI[dcm+]  aciI   apyI[dcm+]        fokI mspA
    apyI[dcm+]         sspI         baeI                  mnlI          eciI  mnlI   sapI          bstF5I ca
901 CCAGGAGGCA GCTGGGGGAA TATTCATCTA TACCCAGGTA TCAATAACCC ATTTCCTCCT GGAGTTCTCC GCCCTCCTGG CTCTTCTTGG AACATCCCAG
    GGTCCTCCGT CGACCCCCTT ATAAGTAGAT ATGGGTCCAT AGTTATTGGG TAAAGGAGGA CCTCAAGAGG CGGGAGGACC GAGAAGAACC TTGTAGGGTC
279 P  G  G  S  W  G  N  I  H  L  Y  P  G  I  N  N  P  F  P  P  G  V  L  R  P  P  G  S  S  W  N  I  P  A

                              rmaI               hgiAI/aspHI
                              maeI               bsp1286
                              bfaI               bsiHKAI
                             styI      tspRI     bmyI
                             bsaJI     btsI      rmaI                              pleI
                             blnI      hpyCH4V   maeI                   bslI       mlyI
                   mnlI      avrII[dam-]         bfaI          mnlI     bslI   bsaXI  hinfI      aciI      mwo
1001 CTGGCTTCCC TAATCCTCCA AGCCCTAGGT TGCAGTGGGG CTAGAGCACG ATAGAGGGAA ACCCAACATT GGGAGTTAGA GTCCTGCTCC CGCCCCTTGC
     GACCGAAGGG ATTAGGAGGT TCGGGATCCA ACGTCACCCC GATCTCGTGC TATCTCCCTT TGGGTTGTAA CCCTCAATCT CAGGACGAGG GCGGGGAACG
313   G  F  P  N  P  P  S  P  R  L  Q  W  G  O
                                    ^59844.AV394.r
                                    ^59844.AV396.er, 5tag=TTTTTTGCGGCCGCTTA
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                    sau96I[dcm-][M.haeIII-]
                    haeIII/palI
                   ecoO109I/draII[dcm-]
                  alwNI[dcm-]
                  alw26I/bsmAI
                 scrFI[dcm-]   pciI
                 pspGI         nspHI
                 mvaI      hpaI nlaIII
                 ecoRII[dcm-]  nspI
                 dsaV[dcm-] tru9I
          bsp1286   bstNI     hincII/hindII
          bmyI      bssKI[dcm-]   aflIII                          mwoI
          banII     apyI[dcm+] mseI                          tspRI mnlI
1101 TGTGTGGGCT CAATCCAGGC CCTGTTAACA TGTTTCCAGC ACTATCCCCA CTTTTCAGTG CCTCCCCTGC TCATCTCCAA TAAAATAAAA GCACTTATGA
     ACACACCCGA GTTAGGTCCG GGACAATTGT ACAAAGGTCG TGATAGGGGT GAAAAGTCAC GGAGGGGACG AGTAGAGGTT ATTTTATTTT CGTGAATACT

                                                                                                              mc
                                                                                                              ea
                                                                                                              ea
                                                                                                              cf
                                                                                                              bs
                                                                                                             not
                                                                                                             fnu
                                                                                                             aci
1201 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAGGGC
     TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTCCCG
```

```
                                scrFI[M.hpaII-]
                                nciI
                                mspI
                                hpaII
                                dsaV                    rsaI
                               xmaI/pspAI         sau96I
                               smaI           mroI     nlaIV
                               scrFI[M.hpaII-]  rsrII/cspI
                               nciI           hpy188III csp6I
                               dsaV           bspMII   kpnI
                      aluI  salI bssKI        bspEI    banI  sfcI
                     sstI   hincII/hindII[M.taqI-] avaII[M.hpaII-]
                     sacI   accI[M.taqI-] tru9I   cpoI  asp718  cac8I
               rmaI  hgiAI/aspHI[M.aluI-] mseI bsaWI cfr10I/bsrFI
               maeI  ecl136II    bsaJI   aseI/asnI/vspI acc65I hpyCH4V
               bfaI  bsp1286[M.aluI-]  xmnI tsp509I  bsaWI    pstI
        aciI  speI   bsiHKAI taqI bssKI tsp509I mspI ageI    sse8387I                    pleI
       fnu4HI/bsoFI  bmyI hpy99I avaI[M.hpaII-] hpaII mspI bspMI      rsaI                mlyI
     haeIII/palI    banII[M.aluI-]    asp700  accIII hpaII  sbfI     csp6I aluI     sfcI   hinfI         aluI
1301 GGCCGCCGAC TAGTGAGCTC GTCGACCCGG GAATTAATTC CGGACCGGTA CCTGCAGGCG TACCAGCTTT CCCTATAGTG AGTCGTATTA GAGCTTGG
     CCGGCGGCTG ATCACTCGAG CAGCTGGGCC CTTAATTAAG GCCTGGCCAT GGACGTCCGC ATGGTCGAAA GGGATATCAC TCAGCATAAT CTCGAACC
```

> length: 1398

aatII(GACGTC):	20
acc65I(GGTACC):	694 805 1347
accI(GTMKAC):	1321
accIII(TCCGGA):	1339

aciI(CCGC): 75 247 254 430 441 490 969 1090 1299 1303
acyI(GRCGYC): 20
afeI(AGCGCT): 453
aflIII(ACRYGT): 32 1128
ageI(ACCGGT): 1344
ahaII(GRCGYC): 20
ahdI(GACNNNNNGTC): 262 580
aluI(AGCT): 14 43 88 203 299 811 844 877 910 999 1316 1365 1392
alw26I(CAGNNNCTG): 1116
alwI(GGATCNNNN): 405 406
alwNI(CAGNNNCTG): 1116
apoI(RAATTY): 49
apyI(CCWGG): 111 130 392 444 520 576 653 689 716 733 802 835 868 901 934 958 976 1115
aseI(ATTAAT): 823 856 889 1333
asnI(ATTAAT): 823 856 889 1333
asp700(GAANNNNTTC): 1331
asp718(GGTACC): 694 805 1347
aspHI(GWGCWC): 1044 1315
avaI(CYCGRG): 57 1326
avaII(GGWCC): 188 261 438 1342
avrII(CCTAGG): 265 1024
baeI(NNNNNNNNNNNNNNNACNNNNGTAYCNNNNNNNNNNNN): 932
bamHI(GGATCC): 405
banI(GGYRCC): 694 805 1347
banII(GRGCYC): 1106 1315
bbsI(GAAGACNNNNNN): 119 528
bbvI(GCAGC): 89 249 375 424 427 456 487 842 875 908
bcgI(NNNNNNNNNNNNCGANNNNNNTGCNNNNNNNNNNNN): 59 752
bciVI(GTATCC): 831 864 897

bclI(TGATCA): 681
bfaI(CTAG): 266 1025 1041 1310
bglI(GCCNNNNNGGC): 971
blnI(CCTAGG): 265 1024
blpI(GCTNAGC): 300
bmyI(GDGCHC): 1044 1106 1315
bpmI(CTGGAG): 480 959
bpu1102I(GCTNAGC): 300
bpuAI(GAAGACNNNNNNN): 119 528
bsaAI(YACGTR): 37
bsaHI(GRCGYC): 20
bsaI(GGTCTCNNNNN): 656
bsaJI(CCNNGG): 129 265 383 409 444 520 623 652 688 689 716 732 733 801 933 1024 1326
bsaWI(WCCGGW): 1339 1344
bsaXI(NNNNNNNNNNNNACNNNNNCTCCNNNNNNNNNN): 146 1072
bseRI(GAGGAGNNNNNNNNNN): 95 163 568
bsgI(GTGCAG): 349
bsh1236I(CGCG): 33 76
bsiEI(CGRYCG): 1300
bsiHKAI(GWGCWC): 1044 1315
bsiWI(CGTACG): 35
bslI(CCNNNNNNNGG): 105 199 313 328 331 364 441 495 502 514 570 576 733 1062 1063
bsmAI(GTCTC): 657
bsmAI(GTCTC): 657
bsmFI(GGGACNNNNNNNNNNNNNN): 187 736
bsoFI(GCNGC): 74 89 249 375 424 427 430 456 487 490 842 875 908 1299 1302
bsp1286(GDGCHC): 1044 1106 1315
bspCNI(CTCAGNNNNNNNNNN): 200 301 401 434 557 770
bspEI(TCCGGA): 1339

bspMI(ACCTGC): 330 1350
bspMII(TCCGGA): 1339
bsrBI(GAGCGG): 254
bsrFI(RCCGGY): 1344
bsrI(ACTGGN): 223 482 615 707 739
bssKI(CCNGG): 111 130 143 392 444 502 520 576 623 653 689 716 733 802 835 868 901 934 958 976 1115 1326 1327

bstDSI(CCRYGG): 383
bstF5I(GGATG): 65 244 663 993
bstNI(CCWGG): 111 130 392 444 520 576 653 689 716 733 802 835 868 901 934 958 976 1115

bstUI(CGCG): 33 76
bstYI(RGATCY): 405
bsu36I(CCTNAGG): 400 556 769
btgI(CCRYGG): 383
btsI(GCAGTGNN): 346 506 1032
cac8I(GCNNGC): 26 30 104 252 305 378 1000 1354
celII(GCTNAGC): 300
cfoI(GCGC): 92 454
cfr10I(RCCGGY): 1344
cfrI(YGGCCR): 1300
cpoI(CGGWCCG): 1341
csp6I(GTAC): 36 695 806 1348 1360
cspI(CGGWCCG): 1341
ddeI(CTNAG): 200 301 401 434 557 770
dpnI(GATC): 406 682
dpnII(GATC): 406 682
draII(RGGNCCY): 729 1117
dsaI(CCRYGG): 383
dsaV(CCNGG): 111 130 143 392 444 502 520 576 623 653 689 716 733 802 835 868 901

	934 958 976 1115 1326 1327
eaeI(YGGCCR):	1300
eagI(CGGCCG):	1300
eam1105I(GACNNNNNGTC):	262 580
earI(CTCTTCNNNN):	10 981
eciI(GGCGGA):	968
ecl136II(GAGCTC):	1315
eclXI(CGGCCG):	1300
eco47III(AGCGCT):	453
eco57I(CTGAAG):	295 461
eco81I(CCTNAGG):	400 556 769
ecoNI(CCTNNNNNAGG):	331 570
ecoO109I(RGGNCCY):	729 1117
ecoRI(GAATTC):	49
ecoRII(CCWGG):	111 130 392 444 520 576 653 689 716 733 802 835 868 901 934 958 976 1115
espI(GCTNAGC):	300
fnu4HI(GCNGC):	74 89 249 375 424 427 430 456 487 490 842 875 908 1299 1302
fnuDII(CGCG):	33 76
fokI(GGATG):	65 244 663 993
gsuI(CTGGAG):	480 959
haeII(RGCGCY):	453
haeIII(GGCC):	73 103 133 226 359 395 492 511 547 730 755 1118 1301
hgiAI(GWGCWC):	1044 1315
hhaI(GCGC):	92 454
hinPI(GCGC):	92 454
hincII(GTYRAC):	1124 1321
hindII(GTYRAC):	1124 1321
hinfI(GANTC):	388 526 580 586 702 776 1079 1380
hinlI(GRCGYC):	20

hpaI(GTTAAC): 1124
hpaII(CCGG): 144 502 623 1327 1340 1345
hphI(GGTGA): 553 684
hpy188I(TCNGA): 46 206 238 294 316 583
hpy188III(TCNNGA): 185 258 534 589 1339
hpy99I(CGWCG): 22 1320
hpyCH4IV(ACGT): 21 38
hpyCH4V(TGCA): 29 68 333 345 350 1031 1353
kpnI(GGTACC): 694 805 1347
ksp632I(CTCTTCNNNN): 10 981
maeI(CTAG): 266 1025 1041 1310
maeII(ACGT): 21 38
mboI(GATC): 406 682
mboII(GAAGA): 10 120 463 529 982
mcrI(CGRYCG): 1300
mluI(ACGCGT): 32
mlyI(GAGTCNNNNN): 526 580 586 1079 1380
mnlI(CCTC): 97 135 163 199 218 230 292 310 338 354 361 403 436 477 495 549 559 570 649 722 728 753 772 839 872 905 955 973 1015 1054 1161
mroI(TCCGGA): 1339
mseI(TTAA): 824 857 890 1125 1334
mspA1I(CMGCKG): 810 843 876 909 998
mspI(CCGG): 144 502 623 1327 1340 1345
mstII(CCTNAGG): 400 556 769
mvaI(CCWGG): 111 130 392 444 520 576 653 689 716 733 802 835 868 901 934 958 976 1115
mvnI(CGCG): 33 76
mwoI(GCNNNNNNNGC): 300 351 424 490 971 1099 1160
nciI(CCSGG): 143 502 623 1326 1327
ncoI(CCATGG): 383

ndeII(GATC): 406 682
nlaIII(CATG): 27 384 759 1129
nlaIV(GGNNCC): 187 226 261 288 395 405 619 694 805 1347
notI(GCGGCCGC): 1299
nspBII(CMGCKG): 810 843 876 909 998
nspHI(RCATGY): 26 1128
nspI(RCATGY): 26 1128
paeR7I(CTCGAG): 57
palI(GGCC): 73 103 133 226 359 395 492 511 547 730 755 1118 1301
pciI(ACATGT): 1128
pflMI(CCANNNNNTGG): 313
pleI(GAGTCNNNN): 526 580 586 1079 1380
pspAI(CCCGGG): 1326
pspGI(CCWGG): 111 130 392 444 520 576 653 689 716 733 802 835 868 901 934 958 976 1115
pstI(CTGCAG): 332 344 1352
pvuII(CAGCTG): 810 843 876 909 998
rmaI(CTAG): 266 1025 1041 1310
rsaI(GTAC): 36 695 806 1348 1360
rsrII(CGGWCCG): 1341
sacI(GAGCTC): 1315
salI(GTCGAC): 1321
sapI(GCTCTTCNNNN): 10 980
sau3AI(GATC): 406 682
sau96I(GGNCC): 72 102 188 226 261 395 438 492 511 730 755 1118 1342
sauI(CCTNAGG): 400 556 769
sbfI(CCTGCAGG): 331 1351
scrFI(CCNGG): 111 130 143 392 444 502 520 576 623 653 689 716 733 802 835 868 901 934 958 976 1115 1326 1327
sfaNI(GCATC): 66 149 538

sfcI(CTRYAG): 5 332 344 1352 1373
smaI(CCCGGG): 1326
smlI(CTYRAG): 57
snaBI(TACGTA): 37
speI(ACTAGT): 1309
sphI(GCATGC): 26
splI(CGTACG): 35
sse8387I(CCTGCAGG): 331 1351
sspI(AATATT): 820 853 886 919
sstI(GAGCTC): 1315
stuI(AGGCCT): 132 546
styI(CCWWGG): 265 383 409 1024
taiI(ACGT): 21 38
taqI(TCGA): 58 1322
tfiI(GAWTC): 388 702 776
thaI(CGCG): 33 76
tliI(CTCGAG): 57
tru9I(TTAA): 824 857 890 1125 1334
tseI(GCWGC): 89 249 375 424 427 456 487 842 875 908
tsp509I(AATT): 50 610 1332 1336
tspRI(NNCAGTGNN): 222 347 483 507 614 708 1033 1156
vspI(ATTAAT): 823 856 889 1333
xcmI(CCANNNNNNNNNTGG): 802 835 868 901
xhoI(CTCGAG): 57
xhoII(RGATCY): 405
xmaI(CCCGGG): 1326
xmaIII(CGGCCG): 1300
xmnI(GAANNNNTTC): 1331

not found:

aclI(AACGTT),aflII(CTTAAG),ahaIII(TTTAAA),alw44I(GTGCAC),apaI(GGGCCC),apaLI(GTGCAC),ascI(GGCGCGCC),aspI(GACNNNGTC),
avaIII(ATGCAT),aviII(TGCGCA),balI(TGGCCA),bbrPI(CACGTG),bceAI(ACGGCNNNNNNNNNNNN),bfrBI(ATGCAT),bfrI(CTTAAG),
bglII(AGATCT),bsaBI(GATNNNNATC),bsiCI(TTCGAA),bsmBI(CGTCTCNNNNN),bsmI(GAATGCN),bsp106(ATCGAT),bsp120I(GGGCCC),
bsp1407I(TGTACA),bspCI(CGATCG),bspDI(ATCGAT),bspHI(TCATGA),bsrDI(GCAATGNN),bsrGI(TGTACA),bssHII(GCGCGC),
bssSI(CTCGTG),bst1107I(GTATAC),bst4CI(ACNGT),bstAPI(GCANNNNNTGC),bstBI(TTCGAA),bstEII(GGTNACC),bstXI(CCANNNNNNTGG),
bstZ17I(GTATAC),btrI(CACGTC),claI(ATCGAT),draI(TTTAAA),draIII(CACNNNGTG),drdI(GACNNNNNNGTC),eco72I(CACGTG),
ecoRV(GATATC),eheI(GGCGCC),esp3I(CGTCTC),fseI(GGCCGGCC),fspI(TGCGCA),hgaI(GACGC),hindIII(AAGCTT),hpyCH4III(ACNGT),
kasI(GGCGCC),kspI(CCGCGG),maeIII(GTNAC),mamI(GATNNNNATC),mfeI(CAATTG),mscI(TGGCCA),mslI(CAYNNNNRTG),munI(CAATTG),
naeI(GCCGGC),narI(GGCGCC),ndeI(CATATG),ngoMI(GCCGGC),nheI(GCTAGC),nruI(TCGCGA),nsiI(ATGCAT),pacI(TTAATTAA),
pflFI(GACNNNGTC),pmeI(GTTTAAAC),pmlI(CACGTG),ppu10I(ATGCAT),ppuMI(RGGWCCY),pshAI(GACNNNNGTC),psiI(TTATAA),
psp1406I(AACGTT),pspOMI(GGGCCC),pvuI(CGATCG),rcaI(TCATGA),sacII(CCGCGG),sanDI(GGGWCCC),scaI(AGTACT),
sceI(TAGGGATAACAGGGTAAT),sexAI(ACCWGGT),sfiI(GGCCNNNNNGGCC),sfuI(TTCGAA),sgfI(GCGATCGC),sgrAI(CRCCGGYG),
snoI(GTGCAC),snoI(GTGCAC),srfI(GCCCGGGC),sstII(CCGCGG),swaI(ATTTAAAT),tsp45I(GTSAC),tth111I(GACNNNGTC),xbaI(TCTAGA)